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The establishment of a novel therapeutic approach in Gliioblastoma multiforme

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Our laboratory has previously established an in vivo syngeneic model for the evaluation of the role of CD95 in glioblastoma progression and/or migration as well as a suitable platform mimicking the development and growing of human glioblastoma tumors. CD95 was found to increase migration of glioma cells by activation of a pro-survival and pro-proliferative signaling pathway downstream of CD95. The connection between the intracellular part of the receptor with Src family kinases upon stimulation with its ligand led to the activation of PI3K and subsequent induction of migration mediated by MMP2 and MMP9 expression. Along this line, the present work represents the logical continuation to those results. Previously the use of antibodies targeting the CD95L has proven to efficiently blocked migration of glioblastoma cells, however the experimental approach followed apart from showing high-variability, was based on the pre-incubation of glioma cells with the antagonist antibodies. This fact was due to the presence of the Blood Brain Barrier (BBB) hampering efficient delivery of high molecular weight proteins into the brain, and accordingly representing a serious obstacle for the potential future development of therapies suitable for systemic administration. Accordingly, in the present work, we sought to target the BBB and induce reversibly opening allowing for delivery of proteins into the brain and so, systemic administration of novel drugs. Thus, during the present studies we tried to develop a novel therapeutic approach for the treatment of Glioblastoma Multiforme. Indeed, our results demonstrate a reversible opening of the BBB, thus we have established and defined a novel experimental setup allowing for therapeutic clinical development and testing of molecular targeting compounds such as recombinant proteins for the treatment of Glioblastoma Multiforme. Moreover, due to the high variability observed when antibodies where employed we decided to design a stable trimer of CD95, the CD95-Fc fusion protein. Altogether, we firstly evaluated the kinetics and the minimum number of cells required for achievement of maximal tumor formation upon injection. To this end we

made use of the established glioma cell line SMA560. SMA cells were stereotactically injected into the left striatum of the Vm/Dk mice and allowed for generation of diffuse and infiltrative glioma tumors. Our results demonstrated that five thousand SMA cells were sufficient to generate 100% of animals bearing tumors. Yet, tumor-bearing animals lived for a longer period of time facilitating the study of the therapeutic effect of CD95-Fc. Increased life-span of the animals allowed for more reliable testing and repetitive treatment in the different therapeutical schemes followed. Indeed, a bigger time-window, alongside slower kinetics of tumor formation, allowed for treatment at their early stage of tumor development. In such a setup, we additionally established a safe way allowing for reversible opening of the Blood Brain Barrier by using High Focused Ultrasound. Reversible opening of the BBB was successfully achieved by application of different HIFU intensities with no observed side-effects on the animals. Furthermore, opening of the BBB contributed to enhanced delivery of the recombinant protein CD95-Fc into the brain and represents a step forward towards the establishment of recombinant protein-based treatments in glioblastoma. We have also evaluated and concisely characterize the relative MRI intensity and half-life of two commonly used contrast agents, Vasovist and Magnevist. Our results demonstrate that Magnevist is a more suitable contrast agent for long-term MRI monitoring and product development leading to a treatment for glioblastoma multiforme. Not only Magnevist presented a longer half-life but also generally higher signal intensities were also observed. Furthermore, apart from the previously described role of CD95 antagonists blocking glioma migration into the contralateral hemisphere, we further characterized this populations of cells and identified glia and Neural/Cancer Stem Cells as the most affected populations after CD95-Fc treatment in vivo. The number of cells present in the contralateral hemisphere expressing GFAP, a glial and “brain” stem cell marker was strongly reduced upon CD95-Fc treatment as compare to the control, indicative of a selective target of these cell populations. Furthermore, we described the potent effect of CD95-Fc as an anti-angiogenic agent in glioblastoma. Angiogenesis and neo vascularization of glioma is a critical step allowing for cell survival and tumor progression, thus blocking of angiogenesis by CD95-Fc might explain the reduced tumor volumes observed as compared to the control, etanercept. Interestingly, recent reports pointed out the ability of Cancer Stem Cells to induce angiogenesis by a transdifferentiation process. Thus, the efficient blocking of angiogenesis due to CD95-Fc administration might be probably due to specific targeting of this population of cells.

Altogether, we have not only established a reliable method for therapeutic product development but most importantly we have set the basics for the generation of high-molecular weight-based treatments, but also demonstrated that reversible opening of the BBB could lead to enhance drug delivery into the brain. Moreover, we have described a novel role for the CD95/CD95L as an angiogenesis blocker. Accordingly, we believe the work here presented can contribute for the future development leading to efficient treatments for Glioblastoma in human patients.